EXHIBIT 8

Predicting bacterial populations based on airborne particulates: A study performed in nonlaminar flow operating rooms during joint arthroplasty surgery

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Background: Prevention of postsurgical infection is preferable to treatment. Prevention requires identification and control of the potential sources of microbial contamination. This study investigated whether the density of airborne particulates can predict the density of viable airborne bacteria at the surgery site.
Methods: A standard particle analyzer was used to measure the number and diameter of airborne particulates during 22 joint arthroplasty surgeries. An impact air sampler and standard culture plates were used to identify and count colony-forming units (CFU).
Results: Particulate density averaged >500,000 particles/m^3 per 10-minute interval, and 1786 CFU were identified, primarily gram-positive cocci. A particle density ≥10 μm explained 41% of the variation in CFU density. Particle and CFU density increased with longer surgery duration and higher staff counts.
Conclusions: These findings support the use of environmental controls that isolate and protect the surgical site from airborne particulates and contamination.
Key Words: Surgical; nosocomial; airborne.

Current estimates indicate that infection occurs in 0.5% to 11% of surgeries, affecting the lives of thousands of patients each year.[1,2] Prevention of infection is preferable to treatment in terms of both patient outcomes and cost of treatment.[3,4] Prevention requires identification and control of the potential sources of microbial contamination.

One potential source of contamination is the air inside the operating room. Studies have demonstrated a correlation between airborne bacterial contamination and postoperative joint sepsis in joint arthroplasty surgery.[5-7] Other studies have addressed the potential for airborne bacteria to result in bacterial deposition in surgical wounds.[8-12] Data on the presence of airborne microbes in the operating room environment, particularly at the surgery site, can be relevant in predicting the risk of infection.

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While measuring airborne bacteria during surgical procedures is not currently feasible, measuring the particulates in the air is relatively simple. Because bacteria compose a portion of the total airborne particulate mass, airborne particulate counts can be correlated with airborne microbial density. The literature regarding the relationship between airborne particulates and airborne microbes is unclear, however; for example, Landrin et al[13] reported no correlation between particle and bacteria counts in operating rooms, but Seal and Clark[14] found a correlation. The study of Landrin et al was conducted in an empty operating room, which does not represent the movements of equipment, operating room staff, and patient typically occurring during orthopedic surgery. The Seal and Clark study data were collected from only 2 actual surgical procedures, calling into question the generality of their results.

The purpose of this study was to determine whether the density of airborne particulates at the surgery site and various behaviors of operating room personnel can be used to predict the density of viable airborne bacteria (ie, colony-forming units [CFU]) at the surgery site during hip and knee joint arthroplasty.

MATERIALS AND METHODS

Approval from the institutional review board at the study institution was obtained before study initiation. Twenty-two patients (10 women, 12 men; mean age 60.0 ± 12.8 years) who had consented to undergo primary hip arthroplasty (6 total and 7 resurfacing) or knee arthroplasty (8 total and 1 unicompartmental) were recruited to participate in this study. Four surgical procedures were chosen that varied with respect to instrumentation, surgical staff, and operating room traffic (eg, for portable radiography during hip procedures), to ensure variability in these factors, which have been identified as possibly related to particulate and CFU counts. Potential subjects were given a written explanation of the study and volunteered to participate by signing an informed consent. Demographic information (age, sex, height, weight, hip vs knee surgery, and comorbidities) was collected for each subject. The surgical procedures were performed between July 1, 2007 and September 19, 2007. All patients received prophylactic intravenous (IV) antibiotics before any skin incisions.

Environment

All air sampling was done during hip arthroplasty and knee arthroplasty procedures performed in 2 operating rooms at the study institution. These are nonlaminar air flow rooms with a conventional ventilation system (turbulent air flow) with a minimum of 15 exchanges per hour. Air passes through a prefilter and a Varicell filter (95% effective at removing particles ≥ 0.3 μm) before being diffused into the room through ceiling vents. Air temperature and humidity are controlled by conventional HVAC methods at set points and approximate ranges of 16°C ± 1°C and 50% ± 7% relative humidity. The operating rooms were kept at a positive pressure level of 0.20-inch water gauge compared with the outer hall and 0.15-inch water gauge relative to the central supply area, to prevent the intrusion of airborne contaminants into the rooms.

The surgeon (G.W.S.) and first assistant (B.T.) wore filtered exhaust helmets and suits during all surgeries. In one operating room, scrub technicians also wore filtered exhaust helmets and suits, but in the other operating room, they did not. Surgical personnel working in the operating room outside the sterile surgical field (eg, circulating nurses, anesthesiologists, radiology technicians, other technicians) wore standard cotton surgical scrub shirts and pants, surgical masks, and hair coverings. These environmental conditions were routine for hip and knee arthroplasty cases performed by this surgeon.

Both operating rooms had 2 entry points via self-closing doors; one door opened to an outer hall, and the other door opened into a central sterile supply area. Access to the sterile supply area was restricted to personnel wearing scrubs, face masks, and hair and shoe covers. The door to the outer hall was locked during surgery to prevent unnecessary traffic, although the door was opened to allow the entrance of radiology equipment.

At the beginning of each 10-minute interval, the number of persons in the room (staff count) was documented, and all entries and exits over the course of the 10-minute interval (traffic flow) were recorded. The duration of the surgical procedure was recorded, as was the specific operating room in which the surgery was performed.

Particulate counts

Airborne particulates were measured using a standard particle analyzer (LASAIR II 310B; Particle Measuring Systems, Boulder, CO) that had been calibrated in May 2007. The particle analyzer sampled continuously throughout the surgical procedure at a rate of 0.0283 m^3/min (1.0 ft^3/min) and logged data at 1-minute intervals to obtain sample volumes of 0.0283 m^3 (28.3 L) of air. The samples were collected through a 100-cm length of sterile Bev-a-line (Thermoplastic Processes, Stirling, NJ) or PVC tubing. The end of the sterile tubing was placed inside the surgical field at a standard location on the "overhead" mayo stand, approximately 40 cm from the surgical wound during hip arthroplasty. Air was drawn through the tube and into the analyzer, where it crossed a laser field. Interruption of the laser field by the particles was detected by an electronic sensor that produced an automated count of the passing particles and measured the diameter of each particle. Particles were classified by diameter (d) in 6 size ranges: $0.3 \leq d < 0.5$ μm, $0.5 \leq d < 1.0$ μm, $1.0 \leq d < 3.0$ μm, $3.0 \leq d < 5.0$ μm, $5.0 \leq d < 10.0$ μm, and/ $d \geq 10$ μm. The count and particle size measures were continuously recorded electronically by the particle analyzer. This information was partitioned into blocks of volume and time that were consistent with the bacterial counting method. Positive hole correction was carried out using tables for 400-hole impactors.

Viable bacteria counts

Airborne viable bacteria counts were measured using an impact air sampler (Anderson N6; Environmental Monitoring Systems, Charlotte, SC). The impact air sampler sampled air at the same rate as the particle analyzer, using a similar collection method and PVC tubing. The sampling end of the tubing was placed adjacent to the particle counter air sampling tube within the sterile surgical field. Air drawn through the tube was passed to a standard culture plate containing

tryptic soy agar with 5% sheep's blood (Healthlink, Jacksonville, FL). Particulate, which included any viable bacteria, collected on the agar surface. The plates were exchanged every 10 minutes throughout the surgical procedure; the exchanging process took approximately 20 seconds. The plates were incubated at 35°C for 3 days. Gram staining and morphological identification were used to identify and count viable bacteria. Viable bacteria were reported as CFU/m^3, a standard unit of measurement for viable bacterial counts. Control plates were handled in the same manner as the test plates, but without exposing them during the surgery. The control plates were used to determine whether handling of the plates contributed to microbial contamination.

Data analysis

Descriptive statistics and data plots were used to evaluate the distributions of the variables. Because the distribution for CFU/m^3 was highly skewed, a square root transformation (sqrt_CFU/m^3) was used to approximate the normal distribution assumed by the linear model. Multilevel (random coefficient) regression analyses were used to analyze the data; this type of analysis is appropriate for longitudinal data with different numbers of data points for each case and inclusion of time-varying covariates (eg, staff count and traffic flow in each 10-minute interval).[15,16] The regression model compared the variation in sqrt_CFU/m^3 within each 10-minute measurement interval to the variations in the predictor variables at each measurement interval while accounting for the dependencies in the data due to the clustering of measurements within surgical cases.

The potential predictors of sqrt_CFU/m^3 included duration of surgery, total particulate count/m^3, the particulate counts in each diameter category, staff count, and traffic flow. The relations between sqrt_CFU/m^3 and each variable were evaluated in separate analyses. A multivariate model was developed to predict sqrt_CFU/m^3. The main effects of each included predictor variable and the respective interaction effects were tested. The models were evaluated by comparing the respective precisions of predicting sqrt_CFU/m^3 (ie, comparing residual variance terms).

RESULTS

We obtained data during 13 hip arthroplasty and 9 knee arthroplasty surgeries, yielding 147 10-minute intervals. Table 1 gives the averages and ranges for the variables included in this study. A total of 1786 CFU were grown in culture. The organisms cultured were 71% gram-positive cocci, 16% gram-positive bacilli, 6.3% gram-negative bacilli, and 7% other, several of which have been associated with postoperative infections following hip and knee arthroplasty. None of the patients developed any clinical signs or symptoms of infection.

Table 1. Means and ranges for measures collected during 13 hip arthroplasty and 9 knee arthroplasty surgeries

Variable	Mean	Range
CFU/m^3 per 10-minute interval	12.5	0 to 93
Surgery duration, minutes	67	48 to 96
Particulate counts in 1000/m^3 per 10-minute interval		
Total (all diameters)	14,425	2972 to 43, 311
Diameter 0.3 to 0.49 μm	12,708	2565 to 36, 562
Diameter 0.5 to 0.99 μm	1333	216 to 7633
Diameter 1.0 to 2.9 μm	319	29 to 2174
Diameter 3.0 to 4.9 μm	39	4 to 243
Diameter 5.0 to 9.99 μm	23	2 to 122
Diameter ≥10 μm	3	0 to 12
Staff count (average per 10-minute interval)	7.9	5 to 12
Traffic flow (operating room entries and exits per 10-minute interval)	5.6	0 to 18

Table 2. Statistical tests of the bivariate associations between each potential predictor variable and the sqrt_CFU/m^3

Variable	Parameter estimate	t	P
Time	−0.016	−2.53	.016*
Particulate count in 1000/m^3			
Total	<0.0001	1.22	.233
0.3 to 0.49 μm	<0.0001	1.21	.240
0.5 to 0.99 μm	0.0001	0.70	.484
1.0 to 2.9 μm	0.0004	0.75	.457
3.0 to 4.9 μm	0.0073	1.93	.056
5.0 to 9.99 μm	0.0156	2.46	.015*
≥10 μm	0.3232	4.65	<.001*
Staff count	0.31	2.62	.011*
Traffic flow	0.034	1.00	.319
Operating room	0.121	1.20	.246

NOTE. The parameter estimates in this table indicate the average change in sqrt_CFU/m^3 per unit increase in the respective variable.
*Statistically significant ($P < .05$).

Table 2 shows the magnitudes of the relationships between each variable and sqrt_CFU/m^3. Neither sex ($P = .267$) nor surgery type (ie, total hip arthroplasty, hip resurfacing, total knee arthroplasty, or unicompartmental knee arthroplasty; $P = .093$) was significantly related to sqrt_CFU/m^3. Surgery duration, 5-μm to 9.99-μm particles/m^3, ≥10-μm particles count/m^3, and staff count were each significantly ($P < .05$) related to sqrt_CFU/m^3.

The regression model including the number of 10-μm particles/m^3 as the only predictor was determined to be the final model predicting sqrt_CFU/m^3

Table 3. Comparison of multivariable models to predict sqrt_CFU/m^3

Model and variables	Parameter estimate	t	P	SEE (CFU/m^3)
Model 1				±8.7
Time	−0.10	−1.17	.254	
5.0 to 9.99 μm	0.011	1.42	.159	
Model 2				±8.4
Time	−0.03	−0.35	.727	
≥10 μm	0.318	4.13	<.001*	
Model 3				±8.4
≥10μm	0.297	4.26	<.001*	
Staff count	0.216	1.88	.064	
Model 4				±8.3
5.0 to 9.99 μm	−0.039	−3.25	.001*	
≥10 μm	0.742	5.15	<.001*	
Model 5				±8.4
≥10 μm	0.325	4.65	<.001*	

NOTE. Particulate counts are in 1000/m^3. Parameter estimate refers to the average increase in sqrt_CFU/m^3 per unit increase in the variable while controlling for the values of the other variables in the respective model.
SEE, standard error of estimation.
*Statistically significant ($P < .05$).

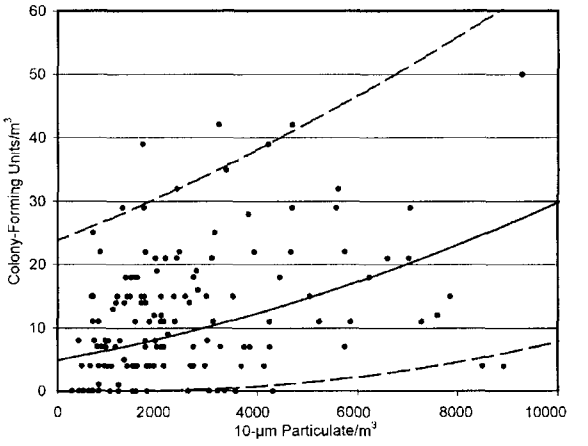

Fig 1. CFU count as a function of 10-μm particulate count. The solid line shows the model-predicted CFU/m^3 using 10-μm particles/m^3. The dashed lines show the 95% prediction interval.

in these data. The 10-μm particles/m^3 accounted for approximately 41% of the observed variation in CFU/m^3 between surgery cases. On average, at least 1 additional CFU/m^3 was detected for every 700 10-μm particles/m^3. None of the other tested models improved prediction accuracy (Table 3).

The precision of predicting CFU/m^3 counts from particulate count was limited. The 95% prediction interval for CFU/m^3 count (ie, after back-transformation of sqrt_CFU/m^3) ranged from ±12 CFU/m^3 at low (<2000) 10-μm particles/m^3 to ±32 CFU/m^3 at high (>8000) 10-μm particles/m^3 (Fig 1).

Table 2 shows that surgery duration and staff count appear to be related to sqrt_CFU/m^3, but these variables were not statistically significant in any of the tested models (Table 3). Follow-up analyses revealed that both surgery duration ($P < .001$) and staff count ($P = .036$) were significantly correlated with the number of 10-μm particles/m^3. Longer surgery duration and higher staff counts were thus associated with both higher sqrt_CFU/m^3 and higher 10-μm particules/m^3. Consequently, the addition of these variables as predictors in addition to 10-μm particles/m^3 failed to improve the prediction accuracy for sqrt_CFU/m^3.

DISCUSSION

The number of 10-μm particles/m^3 and the number of surgical staff in the operating room were associated with the CFU/m^3 at the surgical site during hip and knee joint arthroplasty. The number of surgical staff was correlated with the number of 10-μm particles/m^3. Thus, after controlling for 10-μm particles/m^3, the number of surgical staff was not related to CFU/m^3. A logical interpretation of these data is that increasing surgical staff produces more particulates and more CFUs. Consequently, limiting surgical staff to essential personnel may be a way to control the density of airborne particulates and CFUs in the operating room.

The finding of a correlation between the number of 10-μm particles/m^3 and CFU/m^3 at the surgical site has several important implications. First, it supports airborne particulate contamination of the wound as a source of postoperative infection in joint arthroplasty, as emphasized by Edmiston et al.[17] Second, it lends support to the use of environmental controls in the operating room to limit the number of airborne microbes, such as laminar flow, ultraviolet light, and body-exhaust hoods[18-24] (although it should be noted that a few recent articles have reported that the use of laminar air flow has no apparent affect on postoperative infection rates[25,26]). Third, it suggests that monitoring particulate counts during joint arthroplasty possibly could provide a real-time proxy for increased risk of wound contamination or infection.

We also found a relationship between the number of persons present in the operating room and the CFU/m^3 at the surgery site. This finding is consistent with several previous studies indicating that the source of airborne contaminants in the operating room is surgical and support staff.[18-23] Using bacterial "fingerprinting," one study traced the actual infectious organism in postoperative wound infections to specific members of the operating team.[17] Because the number and behavior of staff present at the surgery table remained relatively constant throughout the study, the activity of persons

in the periphery of the operating room appeared to have contributed to the presence of CFU inside the sterile field at the surgery site. The mean number of personnel in the operating room in each 10-minute interval was 7.9 people (range, 5 to 12). This included a research assistant in addition to the surgeon, first assistant, scrub technician, anesthesiologist (or CRNA) and circulating nurse. One or more sales representatives from implant companies often were present. One or 2 radiology technicians entered and exited the room for portable radiographs during hip arthroplasty. Sometimes a surgical resident, surgical tech student, or additional nurses were present as well. This number of personnel seems high, considering the "limited access" status of the surgical suite. Because particulates and perhaps CFUs may be originating from the peripheral personnel in the operating room, practices that minimize the number of personnel present during surgery may be warranted.

There is no universally recognized standard for acceptable or safe CFU density during surgery. A generally accepted level of airborne microbes for joint arthroplasty is 10 CFU/m^3 in the region of the surgical field.[5,18] In our study of turbulent air flow operating rooms, we measured a mean of 12.5 CFU/m^3 at the surgery site per 10-minute sampling interval. However, a relatively high degree of intraoperative variance existed, with densities ranging from 0 to 93 CFU/m^3. When the density of 10-μm particles in these operating rooms exceeded 3000 particles/m^3 in any 10-minute interval, the average CFU count at the surgical site exceeded 10 CFU/m^3 during that interval.

This relationship between the density of airborne particulate and the presence of viable microorganisms supports the notion that an airborne particle counter may serve as a real-time proxy for airborne bacterial contamination during surgery. Standard practice for detecting and quantifying airborne microorganisms in an operating room is to collect organisms on agar plates using sedimentation or slit sampling methods. The plates are incubated, and CFUs are counted. This method has several disadvantages: (1) It typically takes 3 to 5 days to obtain the results; (2) agar plates typically collect a sample that is remote from the surgical site; and (3) it would be impractical and cost-prohibitive to conduct such monitoring routinely. Commercially available airborne particle counters are portable and provide immediate data on airborne particulate densities, which we observed to be associated with CFU counts at the surgical site. Further studies are needed to validate the use of particulate density to predict the density of airborne microbes.

While our study found a correlation between the number of people in the operating room and the CFUs at the surgical site, no relationship with traffic flow (ie, the number of entrances and exits) was detected. The relationship between CFUs and number of personnel in the OR has been reported by several previous studies.[23,27-30] Some of these studies also have found that traffic flow is related to CFUs. Traffic flow may not have been significant in our study due in part to the relatively high positive pressure in the operating rooms relative to adjacent hallways. The operating rooms had a minimum positive pressure of 0.15 inches of water. The Centers for Disease Control and Prevention recommend at least 0.03-inch water-gauge positive pressure difference between the operating room and adjoining areas.[31] The differential at our facility exceeds this recommendation by several fold, which ay have limited the effects of personnel ingress and egress on airborne particulate.

Bacteria are generally ≥1 μm in size and have a tendency to cluster together and attach to other larger particles. Airborne bacteria-carrying particles measure 4 μm to 20 μm.[32] It is likely that the correlation of larger particles (> 5 μm) with CFUs observed in our study was attributable to the capability of larger particles to carry bacteria. Smaller particles are present in much higher numbers than larger ones, so monitoring particles without discriminating for size ranges obscures identification of the larger particles that may be carrying microbes. This may explain why some previous studies failed to detect a correlation between number of particles and CFUs.

We found that the number airborne particles ≥ 10 μm was correlated with the number of CFUs grown from air sampled within the sterile field approximately 40 cm from the surgical incision. The number of 10-μm particles was associated with the number of staff members present in the operating room during surgery. These observations support the use of environmental controls that isolate and protect the surgical site from airborne particulates and contamination.

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